ANALYTICAL PROFILING OF MEDICINALLY IMPORTANT NATURAL PRODUCTS – CHARACTERISATION OF CUCURBITA SEED OIL

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CERTIFICATE

This is to certify that the dissertation entitled **"ANALYTICAL PROFILING OF MEDICINALLY IMPORTANT NATURAL PRODUCTS – CHARACTERISATION OF CUCURBITA SEED OIL"** submitted by **Ms. KINNERA YEDLAPALLI. (Reg. No: 26101723)** to Tamilnadu Dr. M. G. R. Medical University, Chennai, in partial fulfillment for the award of **MASTER OF PHARMACY IN PHARMACEUTICAL ANALYSIS** at K.M. College of Pharmacy, Madurai, Tamilnadu, is a bonafide work carried out by her under my guidance and supervision during the academic year 2011-2012. This dissertation partially or fully has not been submitted for any other degree or diploma of this University or any other Universities.

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INTRODUCTION

GENERAL INTRODUCTION TO ANALYTICAL PROFILING AND QUALITY CONTROL OF HERBAL MEDICINES

Natural products have a special place in pharmaceutical research. The reasons for interest in natural product chemistry are manifold. First, natural products may serve as lead compounds for new drugs. Second, they give us information on possible biomechanisms and thus on the molecular origin. Third, their isolation has provoked novel analytical and spectroscopic instrumentation and chromatographic techniques such as UV, HPLC, NMR, and Mass spectrometry. Fourth, natural products are permanent challenge with respect to total synthesis and stimulate the development of new reagents and reactions.[1]

While the demand for medicinal plant is increasing their survival in their natural habitats is under growing threats. The primary benefits of using plant derived medicines are that they are relatively safer than synthetic alternatives, offering profound therapeutic and more affordable treatment against various diseases. One of the major problems faced by the modern medicine is the development of resistance by the pathogens to most of the anti-biotics in use. Siddha and Ayurvedic medicines can be effective anti-microbial drugs. Further studies in the field may lead to the development of more medicinal plant extracts which are more effective than modern medicine. [2]

As more natural products are identified as potential lead drug molecules, the demands for more accurate and reliable isolation, extraction and pharmacological evaluation procedures will be felt. Apart from these, standardization of biological and plant materials will have to be developed. As the global consumption of herbal drugs increases, there might be tremendous pressure on the manufacturers and prescribers to not only identify but also quantify the components. Already world organizations like WHO are insisting on analytical profiles of plant drug substances. The example of Aflatoxins and other toxic products content are being regulated. 3, 4

The traditional herbal medicines (HM) and their preparations have been widely used for thousands of years in many oriental countries, such as in India, China, Korea, Japan, etc. However, one of the characteristics of oriental herbal medicine preparations is that all the herbal medicines, either presenting as single herbs or as collections of herbs in composite formulae, are extracted with boiling water during the decoction process. This may be the main reason why quality control of oriental herbal drugs is more difficult than that of western drug. As pointed in general guidelines for methodologies on research and evaluation of traditional medicines (World Health Organization, 2000)5, "Despite its existence and continued use over many centuries, its popularity and extensive use during the last decade, traditional medicine has not been officially recognized in most countries. Consequently, education, training and research in this area have not been accorded due attention and support".

The quantity and quality of the safety and efficacy data on traditional medicines are far from sufficient to meet the criteria needed to support its use world-wide. In general, one or two markers or pharmacologically active components in herbs and or herbal mixtures were currently employed for evaluating the quality and authenticity of herbal medicines, in the identification of the single herb or HM preparations, and in assessing the quantitative herbal composition of an herbal product. This kind of the determination, however, does not give a complete picture of a herbal product, because multiple constituents are usually responsible for its therapeutic effects. These multiple constituents may work 'synergistically' and could hardly be separated into active parts. Moreover, the chemical constituents in component herbs in the HM products may vary depending on harvest seasons, plant origins, drying processes and other factors. Thus, it seems to be necessary to determine most of the phytochemical constituents of herbal products in order to ensure the reliability and repeatability of pharmacological and clinical research, to understand their bioactivities and possible side effects of active compounds and to enhance product quality control.

Thus, several chromatographic techniques, such as high-performance liquid chromatography (HPLC), gas chromatography (GC), capillary electrophoresis (CE) and thin layer chromatography (TLC), can be applied for this kind of documentation. In this way, the full herbal product could be regarded as the active 'compound'. The concept of phytoequivalence was developed in Germany in order to ensure consistency of herbal products. According to this concept, a chemical profile, such as a chromatographic

fingerprint, for a herbal product should be constructed and compared with the profile of a clinically proven reference product. Fingerprints of HM liquid injections are compulsorily carried out for this purpose. In addition, among the various experimental techniques, chromatographic methods are highly recommended for finding out the fingerprints of this products.[6-8]

By definition, a chromatographic fingerprint of a HM is, in practice, a chromatographic pattern of the extract of some common chemical components of pharmacologically active and or chemically characteristics. This chromatographic profile should be featured by the fundamental attributions of "integrity" and "fuzziness" or "sameness" and "differences" so as to chemically represent the HM investigated. It is suggested that with the help of chromatographic fingerprints obtained, the authentication and identification of herbal medicines can be accurately conducted ("integrity") even if the amount and/or concentration of the chemically characteristic constituents are not exactly the same for different samples of this HM (hence, "fuzziness") or, the chromatographic fingerprints could demonstrate both the "sameness" and "differences" between various samples successfully.[9-10]

In general, the methods for quality control of herbal medicines involve sensory inspection (macroscopic and microscopic examinations) and analytical inspection using instrumental techniques such as TLC, HPTLC, GC–MS, LC–MS, near infrared (NIR), and spectrophotometer, etc. On the other hand, the methods of extraction and sample preparation are also of great importance in preparing good fingerprints of herbal medicines. .[11-12]

In the past two decades, combining a chromatographic separation system on-line with a spectroscopic detector in order to obtain structural information on the analytes present in a sample has become the most important approach for the identification and/or confirmation of the identity of target and unknown chemical compounds. For most (trace-level) analytical problems in the research field of herbal medicines, the combination of column liquid chromatography or capillary gas chromatography with a UV–Vis or a mass spectrometer (HPLC–DAD, CE-DAD, GC–MS and LC–MS, respectively) becomes the preferred approach for the analysis of herbal medicines.

With the GC–MS, people could produce not only a chromatographic fingerprint of the essential oil of the herbal medicine but also the information related to its most qualitative and relative quantitative composition. Used in the analysis of the herbal medicines, there are at least two significant advantages for GC–MS, that is: one with the capillary column, GC–MS has in general very good separation ability, which can produce a chemical fingerprint of high quality; two with the coupled mass spectroscopy and the corresponding mass spectral database, the qualitative and relatively quantitative composition information of the herb investigated could be provided by GC–MS, which will be extremely useful for the further research for elucidating the relationship between chemical constituents in herbal medicine and its pharmacology in further research.

In general, one could use the chromatographic techniques to obtain a relatively complete picture of an herbal, which is in common called chromatographic fingerprints of herbal medicines to represent the so-called phytoequivalence. Thus, this is not a simple exercise of applying modern technologies to quality control of the products that have been in constant use for centuries. The progress on quality control of herbal medicines discussed in this review is just at its beginning stage of a long journey. Of course, the proposal of the use of chromatographic fingerprints of herbal medicines for quality control of herbal medicines is definitely a progress.

However, using the chemical fingerprints for the purpose of quality control of herbal medicines can only address to the problem of comparing the integrated sameness and/or difference and controlling their stability of the available herbal products. The complex relationship between the chromatographic fingerprints and efficacy of the herbal medicines is not taken into account yet, which seems to be the most important aspect for the quality control of herbal medicines. [13]

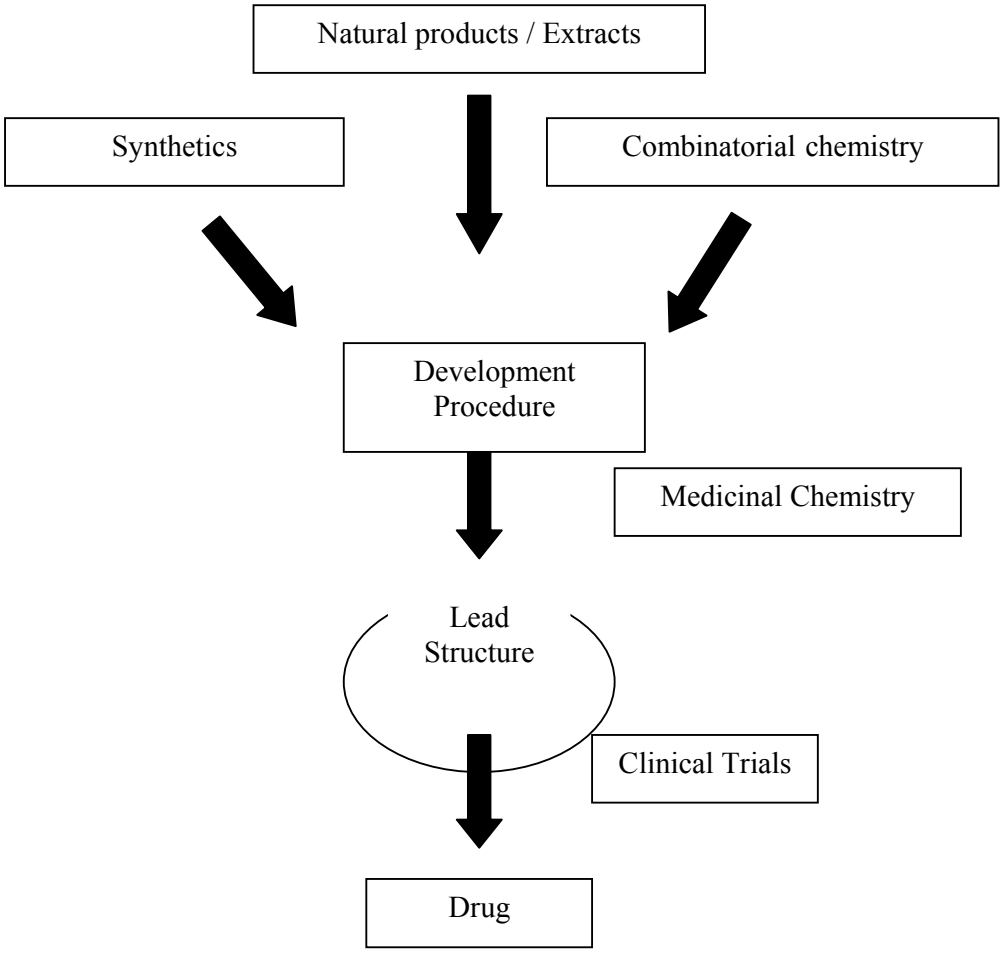
As it is well known that the efficacy of traditional herbal medicines has a characteristic of a complex mixture of chemical compounds present in the herbs, thus how to evaluate reasonably their relationship is obviously not a trivial task. Traditional Herbal Medicines represent a much more daunting challenge due to the natural variability of the individual herbs and the chemical complexity of the formulations. This is where biochemistry, molecular biology, and cell biology are invaluable in establishing quantifiable and reproducible assays. Chemical fingerprints might be linked to these biological assays to

provide assurance of efficacy and consistency. But the research work on this aspect, to our best knowledge, is far from sufficient to meet the criteria needed.

Thus, the researches concerning the relationship between the chromatographic fingerprints and efficacy of the herbal medicines are urgent requirements for the quality control of herbal medicines. On the other hand, the works on possible contaminations in herbal products, such as excessive or banned pesticides, microbial contaminants, heavy metals, chemical toxins, should be also conducted concurrently. In fact, the research field of quality control of herbal medicines is really an interdisciplinary research. It needs crossover of chemistry, pharmacology, medicine and even statistics to provide a platform for the quality control of traditional herbal medicines and further to discover the novel therapeutics composed of multiple chemical compounds.

Different phases in development of approved drug also require standardized analytical procedures and through them a dependable bioanalytical profiling methods can be developed.

SOURCES OF COMPOUNDS FOR DRUG DISCOVERY


Natural products / Extracts
Synthetics
Combinatorial chemistry
Development Procedure
Medicinal Chemistry
Lead Structure
Clinical Trials
Drug

LITERATURE REVIEW

1. Oyebiodun, G. L., et al., (1983) analyzed the proximate, mineral, fatty acid, amino acid and carbohydrate compositions of several parts of the fruit of the fluted pumpkin. The seed contains 53 % fat and 27 % crude protein.[15]

2. Evangelos, S. L., (1986) investigated nutritional and oil characteristics of pumpkin and melon seeds. On a dry basis, the data obtained for the two seeds respectively, were crude oil, 45.4 and 37.8 %; Crude protein, 32.3 and 25.2 %; crude fibre, 12.1 and 15.4 %; ash, 4.65 and 3.85%.[16]

3. Handerson, C. W., et al., (1986) reported trypsin inhibitor, lectin, phytate and oligosaccharides levels in the defatted decorticated *cucurbita* seed meals. Trypsin inhibitor activities in *C. foetidissima* and *C. digitata* samples were 5 times greater than in order cucurbits but were only 17% and 24 % of that in soyabean.[17]

4. Younis, Y. M. H., et al., (2000) examined physicochemical properties and variability in fatty acid composition of the African *Cucurbita pepo* seed oil and reported 4 dominant fatty acids like palmitic (13.3%), stearic (8.0 %), oleic (29.0 %) and linoleic (47.0 %). The oil contains unsaturated fatty acids (78.0 %) and found to be a rich source of linoleic acid (47.0 %). It was concluded that variations were found in seed properties and fatty acid composition of the oil in 3 localities of study.[18]

5. Wenzl, T., et al., (2002) proposed an improved method to discover adulteration of Styrian pumpkin seed oil, included saponification of the triglycerides followed by separation of potassium salts of the fatty acids from the unsaponifiable fraction by adsorption chromatography. It was reported that hydroxyl function of sterol is derivatized with N-methyl-N-(trimethylsilyl) trifluoroacetamide to trimethyl-silyl ether in order to enhance gas chromatographic properties of the analytes, separated on a capillary column of medium polarity (HP 35 MS) in a temperature programmed run within 18 min and detected by flame ionization. It was concluded that this method is on the basis of quantitation of its β-sitosterol content.[19]

6. Applequist, W. L., et al., (2006) compared fatty acid content of seeds of 4 *cucurbita* species grown in a common (shared) garden i.e., *C. maxima*, and *C. argyrospermo* and measured total extractible lipids, fatty acid content in lipophilic extract, palmitic, stearic, oleic and linoleic acid contents by GC-FID. It was concluded that considerable variation was observed in total lipid content and composition among species.[20]

7. Mariod, A. A., et al., (2009) compared the physicochemical properties of six Sudanese cucurbit seeds and seed oils i.e., *Cucumis melo, Cucumis sativus, Citrullius lanatus, Cucumis prophetarum* and *Luffa echinata* conducted proximate analysis for refractive index, relative densities ranges from 1.334-1.442 and 0.874-0.920 gm/cm^3. Unsaponifiable matter ranged between 0.8-1.2 mg KOH/g.[21]

8. Kamel, B. S., et al., (1982) investigated nutritional and oil characteristics of the seeds of angled luffa, *Luffa acutangula* and reported that the kernel has 39 % protein, 44 % fat content and 74.6 % protein content on moisture and fat free basis. Iodine value, saponification value and acid value were found to be 99.5, 190.8 and 10.5 respectively which concludes that the luffa seeds are good source of amino acids, phosphorous, iron and magnesium.[22]

9. Hemavatahy, J., et al., (1992) evaluated lipid composition of melon (*Cucumis melo*) kernel by total kernel lipid extraction and reported that, out of total lipids, 91.5 % neutral lipids, 6.4 % glycolipids and 2.1 % phospholipids were present. Neutral lipids manually 90.7 % triacyl glycerol, 4 glycolipids and 8 phospholipids were detected and their fatty acid compositions were determined.[23]

10. M. Abbas Ali., et al., (2008) have been evaluated the nutritional value of different varieties of *M.charantia* (karela) seed oils.[24]

11. O. A. Abiodun and R. O. Adeleke., (2010) have reviewed nutritional composition through proximate analysis of four varieties of melon seeds.[25]

12. World intellectual property organisation has published details about *L. cylindrica* oil patented for use in skin care cosmetics.[26]

    Patent No. PCT/FR2002/002933

    Pub date : 06/03/2003

13. Ernst L., et al., (2004) reported a complete spectral characterisation of the pumpkin seed oil samples by UV-Visible, NIR and FTIR spectra obtained together with their basic sensorial classification.[27]

14. Seidel, V., et al., (1993) investigated sandwich-type extraction column with on-line sulphuric acid treatment for the determination of organo-chlorine compounds in vegetable oil or oil seeds by gas chromatography with electron capture detection. Organo-chlorine pesticides like hexachloro benzene (HCB), α-hexachloro cyclohexane (HCH), β-HCH, γ-HCH (lindane), heptachlor epoxide, aldrin, 2,4'-DDE, 4,4'-DDE, 2,4'-DDT, 4,4'-DDT and endosulfan can be determined in vegetable oils, using pentachloro benzene and Mirex as internal standards for quantification down to 1-5 ppm level by external or internal standard calibration.[28]

15. Hosamani, et al., (2002) carried out analysis of *Cassia marginata* and *Cassia corymbosa* seed oils by UV, FT-IR, ^{1}HNMR, MS and GLC techniques and found out the fatty acid composition. These seed oils contains vernolic acid (8.5 % and 9.2 %), malvalic acid (3.5 and 3.2 %), sterulic acid (2.6 and 2.8 %), palmitic acid (17.3 and 17.2 %), palmitoleic (trace and 7.4 %), stearic acid (4.5 and 4.2 %), oleic acid (14.2 and 14.8 %) and linoleic acid (49.4 and 41.2 %) respectively.[29]

16. Eronosele, et al., (2002) have found out the fatty acid compositions of seed oils of *Haematostaphis barteri* and *Ximenia americana* by GC/MS technique. *H. berteri* contained 6 fatty acids with oleic (69.35 %) and stearic (15.40 %), the most abundant saturated and unsaturated fatty acids. In *X. americana*, 10 fatty acids were identified of which 7 were unsaturated yielding a total unsaturation of 92.42 %. The oil contained essential fatty acids i.e., linoleic (1.34 %), linolenic (10.31 %) and arachidonic (0.61 %).[30]

17. Cioni, et al., (2005) have carried out analysis of volatile fraction, fixed oil and tegumental waxes of the seed of two different cultivars of *Helianthus annuus* by GC and GC/MS. They have observed that γ–Pinene, cis-verbenol and β-gurjunene were the main volatiles but found with significant quantitative differences. Moreover, florom oil was characterized by a greater variety of constituents.[31]

18. Ramadan, et al., (2006) characterized fatty acids and bio-active compounds of kachnar (*Bauhinia purpuria*) seed oil by CC, GC, TLC and normal-phase HPLC and found out the amount of neutral lipids in the crude seed oil was highest (99 % of total lipids), followed by glycolipids and phospholipids.[32]

19. Bravi, E., et al., (2006) developed HPLC separation and evaporative light scattering detection (ELSD) for quantitative analysis of fatty acid methyl esters (FAME) in 3 different oils i.e., rice bran oil, pumpkin seed oil as reference standard. It was concluded that HPLC coupled with ELSD allows good separation of FAMEs in oil sample and this procedure is an excellent alternative to traditional methods for fatty acids analysis.[33]

AIM OF PRESENT STUDY

Analysts have a thirst for the search of newer analytical methods which may be superior to the present available methods. The evolution of the drugs varies in period of about five decades. Challenges exist for the development of superior analytical methods. In spite of their diversified pharmacological actions, drug substances have unique physical and chemical properties which opened a gateway for their quantification.

The plants chosen for the study are *Cucurbita maxima*. A review of literature reveals that there were very few reports on chemical contents and utility parameters for *C. maxima* seed oil. The present work is focused on development of analytical profile for seed oils and selected medicinal properties.

PLAN OF WORK

1. Collection and identification of the plant material
2. Extraction of oil
3. Evaluation of physical constituents
    - ❖ Description
    - ❖ Solubility
    - ❖ Emulsification index
    - ❖ Specific gravity
    - ❖ Refractive index
4. Estimation of chemical constituents
    - ❖ Acid value
    - ❖ Iodine value
    - ❖ Saponification value
    - ❖ Peroxide value
5. Analysis of the oil by

- ❖ IR-spectroscopy
- ❖ Preparation of methyl esters
- ❖ GC/MS
- ❖ Villavecchia test

6. Preparation of fruit and seed coat for screening.
7. Free radical scavenging activity.

   Two methods – DPPH scavenging method
   – Nitric oxide scavenging method.

PLANT PROFILE

INTRODUCTION OF MEDICINAL PLANTS

According to Ayurvedic and Unani system of medicine's literature and prescription by the practitioners, one hundred and seventy-five plants belonging to 65 different families alleged to possess medicinal properties, were collected with suitable botanical considerations and physiological activities. Preliminary qualitative chemical analysis was carried out at the same time.

Sixty-four of these plants were found to be physiologically active, 29 showing multiple activities and the other 35 plants single specific activity. The remaining 111 plants were inactive and did not respond to the tests employed.

Although herbal medicine plays a role in the life of the people of almost every part of the world, the systematic study and the use of plant materials as chemotherapeutic agents for centuries in India hardly finds its counterpart anywhere else. From years of experience of the flora of this country, the original inhabitants, and many others who came and settled here, developed the Ayurvedic and thereafter the Unani system of Indian medicine.

There is little doubt that today the Ayurvedic and Unani systems of medicine provide the most widely used therapeutic measures for the masses of India. Indian medicinal plants go into the preparation of dozens of Ayurvedic and Unani pharmaceutical specialities which are popular and utilised extensively.

During recent years these two systems of medicine are being given a great deal of attention. Efforts are being made to popularize, propagate and modernize this storehouse of clinical and other observations. Boards and faculties of Ayurvedic and Unani systems of medicines are being set up. Research by national laboratories and other institutions is encouraged, with the object that herbal flora of the country be analysed by present-day chemical, pharmacological, microbiological, clinical and other procedures, so that pure chemical principles of proved therapeutic value be isolated and included in our pharmacopoeia and National Formulary. In this connection, mention has to be made of the stimulus which this branch of study received during the present decade by the discovery of reserpine from the well known Indian medicinal plant *Rawolfia serpentina* Benth., with its multiple physiological activities, by Schlittler and his collaborators.

In various samhitas and modern texts on the subject, the medicinal properties of 800 – 1300 plants are described. Screening of 175 well-known Indian medicinal plants for their

biological activity from the point of view of antibacterial, antitubercular and antifungal actionhas been reported.[55]

INTRODUCTION TO CUCURBITACEAE

This family is known to contain several bioactive compounds such as cucurbitacins, triterpenes, sterols and alkaloids. Plants containing cucurbitacins were early recognized in folk medicine to have biological values. Moreover, many plants used in folk medicine to treat inflammatory conditions have been found to contain cucurbitacins displaying potentially important anti-inflammatory actions, in different invivo and invitro assays.

The attractive association between chronic inflammation and incidence of many diseases has been fertile land for the growth of ethnobiomedicinal research. Traditionally, plants have been used to treat various human diseases including inflammation. Cucurbita plants were used actively as traditional herbal remedies for various diseases. Cucurbita plants demonstrated anti-inflammatory, antitumor, hepatoprotective and immunoregulatory activities.

The plant family Cucurbitaceae is composed of about 850 species and 90 –100 genera, distributed throughout the world. A search of the literature revealed that most of the phytochemical screening is in the area of chemical constituents often referred to as bitter principles, which are presents in about 64 species belonging to 30 genera. These principles were found in various parts of the plants, viz fruits (37 sps, 9 genera) roots 24 sps, 18 genera) and leaves (8 sps, 6genera) according to Rehm and Enslin 1957.[34] Many of these are reputed to possess medicinal or toxic properties. Most of the substances described in the older literature were badly characterized and may be mixtures of many compounds.

Water melon (*citrullus lanatus* var. citroides; belongs to cucurbitaceae family) is a low climbing, hairy and annual plant. In Northern sudan, this plant is often used for rheumatism, swellings, gout and as laxative.[35]

The root extract of *wilbrandia ebracteata* (cucurbitaceae) a plant commonly used in Brazil to treat rheumatic disease was reported to contain several cucurbitacins.

Aliphatic constituents, triterpenes, sterols

Fruits and seeds

The crystalline bitter principles from the plants belonging to the family Cucurbitaceae are named cucurbitacins (1) A, B, C, D and E (2, 3, 4 & 5 a & b). The amorphous forms from certain species were found to contain glycosides which yielded crystalline bitter principles on hydrolysis with a specific enzyme elasterase. Most extensive investigations were carried out on Cucumis and Citrullus plants. Their structures and chemical properties were established. (Enslin 1957).[36]

Only a few, well defined substances have been isolated. Some of them are α-Elaterin isolated from *Citrullus colycynthis* attracted most attention due to its medicinal properties (Enslin, 1954).[37] The crystalline bitter principles isolated from *L. cylindrica, L. acutangula and L. echinata* of mp 182-184 are most probably the same. The search for these principles in other genera, especially *Luffa* was largely the result of isolation of a crystalline principle by Chaudhary et al., (1959) from *L. amara* which they named as amarin.[38] Then followed a spate of reports on these compounds. The isolation of crystalline bitter principle from *L. aegyptica.* by Rangaswami and Sambamurthy, 1954.[39]

They also reported its toxicity on fresh water fish. Chemical examination of *L. acutangula* by Barua etal 1958, led to the conclusion that amarin could be cucurbitacin B. They also reported the isolation of an acid sapogenin oleanolic acid. This conclusion was based on comparing the chemical and physical data of this with cucurbitacin B characterized by Rivett and Herbestein, 1957.[40] Varshney and Khan, 1960 working on the same plant isolated and characterized the sapogenins and reported that this plant contains an unidentified neutral genin.[41]

The occurrence of some variants like epi-cucurbitacins (6) from *L. echinata* by Lavie 1962 [42] which lacked the double bond in the side chain also threw some light on the possible structure of cucurbitacins (Varshney & Khan, 1965). [43]

Varshney and Beg 1977 reinvestigated *L.aegyptica* and could characterize the saponins and sapogenins and designated them as Aegyptinin –A and Aegyptinin –B. These were containing genins oleanolic acid and gypsogenin[44.]However most of these compounds are yet to tested for pharmacological activity.

Amino acid and protein content

Cucurbita seeds are reported to contain about 35% of crude protein. The amino acid composition of seeds of the Cucurbitaceae family was reviewed by Patrica 1965.[45] It is interesting to note that some of the species contained rare amino acids like N-methyl asparagines (9), N-hydroxymethyl asparagine (10), which were showing anthelminthic activity (D.O Grey 1961).[46] The isolation of cucurtbitin (11) (3-aminopyrrolidine-3-carboxylic acid) and carboxy phenylalanine (12) were reported for the first time in higher plants. The heterocyclic amino acid β-pyrazol-1-yalanine (13) isolated from watermelon seed showed high cytotoxic activity. (Akjaer and P.O.Larsen, 1963).[47]

Flavanoids and other Phenolics

L.Cylindrica (the alcoholic extract of whole plant) was investigated for the presence of bio flavanoids by Ganju and Puri 1959.[48] They have reported that these species do not contain bio flavanoids. Seshsadri and Vydeeswaran, 1971 [49] however reported on chemical examination of *L. echinata*, the presence of not only common flavanoids like luteolin and isoplumbagin (14 & 15) but also a rare flavanoid chrysoeriol (16) along with its 7-glucoside and 7-apioglucoside. The 7-apioglucoside has been reported from Cucurbitaceae for the first time.

Oils and Fats

Many Cucurbitaceae produce seeds rich in oil and protein. Although none of these oils has been utilized on an industrial scale, many are used as cooking oil in some countries in Africa, and the Middle East. The seeds of *L. graveolens* contained an oil in which the fatty

acid are stearic and linoleic acids (Seghal 1961).[50] The nutritional significance and oil characteristics were mainly reported from the seeds of pumpkin and melon. On dry basis the two seeds contained 45% and 35%, crude oil and crude protein respectively. (Lazos 1986).[51] There are no specific investigations on *L.cylindrica* seeds.

Mineral and Vitamin content

The fruits of *L. acutangula* and *L.cylindrica* were investigated for these constituents. *L. cylindrica* on comparison was found to be superior with respect to thiamine, riboflavine, niacin, carotene and calcium. While *L. acutangula* had the higher content of vitamin C and iron. (Gopalan 1984).[52]

Vit E content in pumpkin seeds were reported by Murkovic et al., (1996).[53]

Biological and Pharmacological activity survey

The plant has been studied for antibacterial, antifungal, antiviral, immunoinhibitory, anticancer and also antidiabetic effects. A brief review of the available information on the various biological and pharmacological activities is presented here. It is important to mention here the fact that most of the reported pharmacology work is from relatively few genera like Citrullus and Gynostemma. The information regarding the activities of the Cucurbita species is mainly discussed. Morphologically most of the screening was from the roots and the fruit. Against this background we have directed our work towards seed extracts.

The literature available on the enzyme inhibitors and other anti-nutritional factors is also presented. This area of work may reveal the actual medicinal potential of the Cucurbitaceae as a source of lead compounds for future drug design. This aspect is discussed in some detail in the enzyme inhibitory activities chapter.

C. maxima extracts had a significant action on trematodes (Fasciolopsis buski) but not on nematodes (Ascaris lumbricoides) in the invitro studies.[54]

The fruit pulp of *C. pepo* was found to be highly nutritive since all important nutrients like proteins, carbohydrates and minerals were found to be present.

Systemic Effects

Cephalandra indica leaves have been reported to possess spasmodic and uterine motility action.[55] Fruits of oil pumpkin were reported to have inhibited cough reflex.[56]

The earlier reports on the alcoholic extracts of different *Luffa* plants did not show any CNS, CVS and diuretic activity (Aswal 1984).[57] The aqueous extracts of *L.echinata* fruits significantly lowered bilirubin levels in chlorpromazine induced jaundiced rats (Bapat & Chandra1968).[58] The alcoholic and ether extracts of the whole plant showed definite protection against carbontetrachloride induced liver injury in rats. The ether extract exerted significant stimulation of isolated guinea pig ileum (Lauria 1972).[59] The therapeutic potential of *L.echinata* has been reviewed by Vaidya and Antarkar 1984. The alkaloid obtained from the fruits of *L.echinata* showed hypotensive action as also cardiac depressant effect on isolated heart preparations. This compound has exhibited a significant local anaesthetic action. (Gajaria etal 1978).[60]

Anti inflammatory activity

This has been confirmed in the alcoholic extract of the leaves and roots of certain species of Cucurbitaceae. This activity was attributed to iso-plumbagin among the various phytoconstituents, which was found to have activity similar to phenylbutazone (Peters,1997).[61] Activity is also reported from wild melon *citrullus lanatus* and active principle has been identified as Cucurbitacin E.

Anti Parasitic and Insecticidal activity

The different extracts of the various Luffa species did not show any insecticidal activity. On the contrary, it has exhibited growth promoting features (Atal 1978).[62] The specialised amino acids viz non protein aminoacids like methyl asparagines and hydroxy asparagines from certain Cucurbita plants like *Bryonia diocia* have shown considerable anthelminthic activity.[45] citrus medica L oil have antifungal effect on fungi of Ascharis hypogea.[63]

Anti microbial activity

The crude aqueous extract of the riped fruit showed on invitro studies that the extract inhibited growth of the viruient strains of Mycobacterium tuberculosis.[64]

The juice of *L. acutangula, L. cylindrica and L. echinata* has been tested for anti fungal activity against. Seeds of *L. cylindrical* have shown considerable activity against pathogenic fungi like Cephalosporium sacchari., while other plants juices were found mildly active. (Dixit&Tripathi 1975).[65] The ethanolic extract of the seeds of *L. cylindrica* had more than 80% fungitoxic activity against Helminthosporium oryzae (Dixit etal 1978).[66] *L.graveolens* did not show any anti fungal activity. (Bhakuni 1971).[67]

Anti cancer activity

In a search for anticancer drugs from the Indian medicinal plant S.K.Pal et al., 1968 have screened a certain group of plant or rather "Natural Orders" whose members have shown in recent years as having significant antineoplastic activity[68]. In this survey *L. cylindrica* and *L. acutangula* were most promising. In separate investigations Gitter et al., 1961 have reported that plants belonging to the Cucurbitaceae possess significant anti tumour activities[69]. A new series of compounds called cucurbitacins S_1 and S_2 have been isolated from *Bryonia dioica.* These highly cytotoxic principles are alpha ketols in ring A of their structure (7,8) (Kupchan etal).[70]

Enzyme Inhibitors

Tryspin inhibitors characterised from the seeds of *C. maxima* were considered to belong to a new family of serine protease inhibitors. They were unique for many reasons, because of their small size (about 30 amino acid residues) (Polanowski 1980)[71], a strong inhibitory activity and high cell compact structure such polypeptides are very attractive templates to design inhibitors for physiologically important enzymes (Wilusz et al., 1983)[72]. Some of the synthetic variants of these natural inhibitors while losing their inhibitory tryspin activity have become a strong human leukocyte elastase inhibitors (HLE) (Hara et al., 1989)[73]. Lately a lot of interest in HLE inhibitors is being shown because of their potential use as therapeutic agents in several diseases like e.g. rheumatoid arthritis, respiratory distress syndrome, asthma and other inflammatory disorders (Rozycki etal 1995).[74]

The research group at the Institute of Biochemistry, University of Wroclaw, Poland have so isolated about 14 such inhibitors and using their structures as lead compounds, synthesized more than 40 analogues with varying degree of trypsin, chymotrypsisn and elastase inhibitory activities (Rozycki etal 1995).[75] The presence of such a class of inhibitors has been reported only in two families Apocynaceae and Boroginaceae (Wieczorek, etal 1985).[76]

Amylase and Urease inhibitors have been studied in Cucurbita and Citrullus plants only (Malhothra 1978).[77] Amylase inhibitors and other anti nutritional factor like lectins, phytates were investigated to evaluate seed meals of xerophytic species as potential food

sources with respect to inherent levels of these nutritional antagonists (Henderson 1986).[78] *C. pepo* seed protein have shown hepatoprotective action.

PLANT PROFILE OF *CUCURBITA MAXIMA*

Botanical name - *Cucurbita maxima*

Genus - Cucurbita

Family name - Cucurbitaceae

Synonyms

English	-	Pumpkin
Gujarati	-	kolu
Hindi	-	kaddu
Kannada	-	kumbalakai
Marathi	-	Lal bhopala
Tamil	-	Poosanikai
Telugu	-	Gummadikai
Bengali	-	Saphurii
Malayalam	-	Chakkera Kumpalan

Fig no. 1 *Cucurbita maxima fruit*

Fig no. 2 *Cucurbita maxima* flower and leaves

Fig no. 3 *Cucurbita maxima* seeds

Fig no. 4 *Cucurbita maxima* oil

Description

Pumpkin is a gourd-like squash of the genus Cucurbita and the family of

Cucurbitaceae.[79]

The word pumpkin originates from the word pepon which is Greek for “large melon”. Pumpkins are a squash-like fruit that range in size from less than 0.45 kg to over 450 kgs.[80] Pumpkin stems are more rigid, prickly, and angular.[81, 82] Fruit varies greatly in shape, ranging from oblate to oblong.[83] The rind is smooth and usually highly ribbed.

Pumpkins are monoecious, having both male and female flowers on the same plant. The female flower is distinguished by the small ovary at the base of petals. The colour of pumpkin is derived from the orange pigments abundant in them.[84]

Distribution

Biggest international producers of pumpkins include the United states, Canada, Mexico, India and China.[85]

Parts used

Seeds and fruits.

Uses

Cooking

Pumpkin are very versatile in their uses for cooking. Most parts of pumpkin are edible, including thee fleshy shell, the seeds, the leaves, and even the flowers.[86]

Raw Pumpkin

Table no. 1: Nutritional value per 100 gm

Energy	13kcal	Niacin(vit B_3)	0.6 mg (4%)
Carbohydrates	65 gm	Pantothenic acid	0.298 mg (6%)
Sugars	1.35 gm	Vitamin B_6	0.061 mg (5%)
Dietary fiber	0.5 gm	Folate(vit B_9)	16 µg (4%)
Fat	0.1 gm	Vitamin C	9 mg (11%)
Saturated	0.05 gm	Vitamin E	1.06 mg (7%)
Monosaturated	0.01 gm	Calcium	21 mg (2%)
Polyunsaturated	0.01 gm	Iron	0.8 mg (6%)
Protien	1.0 gm	Magnesium	12 mg (3%)
Vitamin A	369 µg (46%)	Phosphorous	44 mg (6%)
Beta-carotene	3100 µg (29%)	Potassium	340 mg (7%)
Thiamine(vit B_1)	0.05 mg (4%)	Sodium	1 mg (0%)
Riboflavin(vit B_2)	0.11 mg (9%)	Zinc	0.32 mg (3%

Traditional uses or Folklore

Extract

East China Normal University research on type-1 diabetic rats, published in July 2007, suggests that chemical compounds found in pumpkin promote regeneration of damaged pancreatic cells, resulting in increased bloodstream insulin levels. According to the research team leader, pumpkin extract may be "a very good product for pre-diabetic people, as well as those who already have diabetes," possibly reducing or eliminating the need for insulin injections for some type-1 diabetics. It is unknown whether pumpkin extract has any effect on diabetes mellitus type 2, as it was not the subject of the study.[87]

Pulp

Decoctions from the pulp are used to relieve intestinal inflammation or applied as a poultice or plaster to burns.

Seeds

Seeds are small, flat, green and edible. They are good source of protein, phytosterols, and vitamins. Used as vermifuge particularly against tapeworms. Seeds are high in

magnesium, manganese, phosphorous, zinc have been recommended in the early stages of prostate problems.[88]

Seeds are diuretic and used as a treatment for nephritis and other urinary system problems.

Seed oil

Long believed to be a folk remedy for prostate problems, it has been claimed to combat benign prostatic hyperplasia.[89] Seed oil contains essential fatty acids that help to maintain healthy blood vessels, nerves and tissues.[90]

Other uses

The medicinal properties include anti-diabetic, anti-oxidant, anti-carcinogenic and anti-inflammatory.[91] Canned pumpkin used as dietary supplement for dogs and cats as digestive ailment such as constipation and diarrhea.[92] Raw pumpkin can be fed to poultry for egg production in cold seasons. Pumpkin acts as antirheumatic, a demulcent, a nervine and a taenifuge. Pumpkin is also used to treat boils, carbuncles, fever, measles, skin ailments, small pox, sprains, tumor, urinary ailment, warts and women's ailments.

Chemical constituents

Amino acids : Alanine, Arginine, Cucurbitin, Cystine, Glycine, Histidine, Isoleucine, Lysine
Acids : Linoleic acid, Aspartic acid, Oleic acid, Palmitic acid
Vitamins : Vit.A, Vit.B
Fats : Lecithin
Minerals : Calcium, Cobalt, Boron, Magnesium, Zinc, Potassium, Iron
Sugar : Sucrose

PLANT COLLECTION

Cucurbita maxima

The plant materials used in the present study were fully ripened matured seeds from characterized plants of *C. maxima* were collected from several plants from the horticulture centre T.N. Agricultural University Madurai. The plant has been authenticated by Dr. D. Stephen, PG Department of Botany, American college, Madurai (Letter enclosed). The seeds were separated from the fruits and were shade dried for 3 days. The dried seeds were made into a coarse powder and were used for extraction procedures. The main focus is on extraction, purification and characterization of the seed oil.

Extraction of oil

Dried coarse powder of the seeds (250 gms) of C. *maxima* was used in portions of 40 gms placed into the extractor of a soxhlet apparatus and subjected to extraction by hot percolation method. The extraction was carried out using 2 litres of petroleum ether for a period of 24-30 hours. The extract was concentrated by using rotary flash evaporator. The oily extract was transferred to a watch glass and placed in a desicator containing fused calcium chloride.

Separation of oil

Column chromatography

The petroleum ether extract obtained as a gummy mass was loaded on to a silica gel column and n-hexane has been used as mobile phase.

Preparation of the column

A glass column of 3.2 cm diameter was packed with activated silica gel in the form of slurry in petroleum ether. The column was packed to a height of 45.0 cm in order to establish a column volume of 375.0 ml.

The column was developed according to the following lines. The column was conditioned up by passing two column volumes of petroleum ether before the residue was loaded. The solvent was kept 5cms above the bed and the residue was carefully loaded in the

form of petroleum ether slurry. The column was then developed with n-hexane. Fractions of 40 ml were collected and solvent evaporated to get golden yellow oil. The defatted seed has been processed for pharmacological investigations.

Yield of the oil

The yield of the *C. maxima* seed oil is of about 24.61%.

Identification

1. **TLC**

Mobile Phase	:	Hexane: chloroform: ethyl acetate 7 : 2.8 : 0.2
Detection	:	Iodine chamber Formation of spots

2. **Filter Paper Test** : Translucent spot

MATERIALS & METHODS

Instruments used

1. GC Clarus 500 Perkin Elmer, USA.
2. Double beam UV-Visible spectrophotometer- Perkin Elmer EZ - 301, USA.
3. FT-IR spectrometer, spectrum RX1, Perkin Elmer, serial no: 69751, part no: 11185247, USA.
4. Hydraulic pellet press- type KP, serial no: 496, Kimaya Engineers, Thane, India.
5. Rotary vacuum flash evaporator- Equitron, Mumbai.
6. Refractometer - Advance Research Instruments Company, C-22, DDA sheds, Okhla, Phase-1, New Delhi.
7. UV chamber: TLC spot detector - Labline, Kochin
8. Kemi hot air oven- Model: KOS.3, Temp-550 °C, Volts-220/230 AC, Kadavil Electro Mechanical Industries, Ernakulam, Kerala.

9. Electronic balance - Shimadzu Corporation, Shimadzu Philippines Manufacturing Inc. (SPM)
10. Heating mantle - Sigma Instruments, Chennai.

Chemicals used

1. Diethyl ether, potassium hydroxide, potassium hydrogen phthalate, pyridine, sodium carbonate, silica gel and acetic anhydride (S. d. fine Chem. Ltd., Mumbai).
2. Carbon tetrachloride, sodium thiosulphate and methyl red (Universal Laboratories Pvt. Ltd., Raheja Centre, Nariman point, Mumbai, India).
3. Potassium bromate and phenolphthalein (Qualigens Fine Chemicals, A division of Glaxo India Ltd., Dr. Annie Besant Road, Mumbai).
4. Absolute alcohol (Hayman Ltd., Essex, CM83YE, England)
5. Potassium iodide (Merck Ltd., Mumbai).

All the solvents used were of AR grade unless otherwise specified. Glass distilled water was used throughout the analysis.